Remarks

Election/Restrictions

Claims 1, 2, 7, 12, 19, 24, 26-28, 33-34, 49, and 51 are currently under consideration. Claims 3-6, 8-11, 13, 35, 38, and 45 have been cancelled. Claims 14-18, 20-23, 25, 29-32, 36-37, 39-44, 46-48, 50, and 52-110 have been withdrawn. Claim 19 is currently amended. Claims 111-116 are new.

Information Disclosure Statement

The Examiner states that he has not received copies of the references filed with the Information Disclosure Statement mailed July 22, 2003. Applicants note that copies of all 62 references cited in said Information Disclosure Statement were, in fact, mailed to and received by the U.S. PTO as indicated by the date stamped postcard returned from the U.S. PTO on July 25, 2003. For the Examiner's convenience, however, duplicate copies of said references were hand delivered to the Examiner on July 31, 2003.

Specification/Informalities

The Examiner states that the title of the invention is not descriptive and suggests a new title. Applicants traverse. It is Applicants' position that the title is sufficiently descriptive. The Examiner's suggested title is unnecessarily limiting in that it recites "overexpressing" where, in fact, the invention relates to other microorganisms (*i.e.* having deregulated pantothenate biosynthesis enzymes) for use in the claimed methodologies. Moreover, the present title is consistently used to describe the invention worldwide. In view of the above Applicants request withdrawal of the objection.

Claim Rejections

Applicants note the claim objections in paragraphs [8] and [9] of the instant Office Action. For the purpose of Examination, the Examiner's reading of the claims is sufficient. However, Applicants note that upon allowance of a generic claim, Applicants will be entitled to consideration of claims to additional species which are written in

dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR 1.141. Accordingly, no amendment of claims 24 or 49 is being made at this time.

Rejection of Claims 19, 24, 26, 27, 33, 34 and 49 Under 35 USC §112, Second Paragraph

Claims 19, 24, 26, 27, 33, 34 and 49 have been rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention (paragraph [10]). Applicants note that claims 1, 2, 7, 12 and 51 are not included in this rejection but are discussed in subpart [a.]. Applicants assume the Examiner intended to recite claims 1, 2, 7, 12 and 51 at paragraph [10], but requests clarification if this understanding is incorrect.

The Examiner states that claims 1, 2, 7, 12 and 51 are "unclear in the recitation of 'panto-compound'" and [w]hile Figure 1 shows a pantothenate biosynthetic pathway and intermediates thereof, there is no indication in the specification, claims or figures as to those compounds of the pantothenate biosynthetic pathway that are considered to be 'key compounds'" [10a.]. Applicants traverse. Contrary to the Examiner's assertion that "key compounds" are not indicated, the specification defines the term 'panto-compound' as including a compound (*e.g.*, a substrate, intermediate or product) in the pantothenate biosynthetic pathway which is downstream from a particular pantothenate biosynthetic enzyme". Page 14, lines 10 to 13, teaches exemplary panto-compounds including ketopantoate, pantoate, β-alanine and pantothenate are 'panto-compounds'. The specification further describes panto-compounds as those that can be produced by manipulating at least one enzyme of the pantothenate biosynthetic pathway (page 12, line 10), by having at least one valine-isoleucine biosynthetic enzyme described herein, manipulated (page 12, lines 17 to 18), by having at least one of acetohydroxyacid synthetase, acetohydroxyacid isomeroreductase or dihydroxyacid dehydratase manipulated (page 12, lines 21 to 22), by culturing a ketopantoate reductase-overexpressing (KPAR-O) microorganism (page 12, lines 25-26), by culturing an aspartate-α-decarboxylase-overexpressing (AαD-O) microorganism (page 12, lines 27-

28), or by utilizing microorganisms having modified pantothenate kinase activity (page 12, line 35).

Applicant respectfully submits that the essential inquiry is whether the claims set out and circumscribe a particular subject matter with a *reasonable* degree of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but rather is to be analyzed in light of the content of the particular application disclosure, the teachings of the prior art, and the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. Applicant respectfully submits that the phrase "panto-compound when read in light of the instant specification and in view of the pertinent skill in the art is both particular and clear. In view of the foregoing comments, Applicants respectfully request reconsideration and withdrawal of any rejection of claims 1, 2, 7, 12 and 51 under 35 USC §112, second paragraph as being indefinite.

The Examiner also rejects claim 19 for the use of the term "significantly high yield" as being a "relative term which renders the claim indefinite".

The term "significantly high yield" is defined on page 30 (lines 1 to 19) of the specification as "including a level of production or yield which is sufficiently elevated or above what is usual for comparable production methods, for example, which is elevated to a level sufficient for commercial production of the desired product (*e.g.*, production of the product at a commercially feasible cost)". To further clarify the meaning of the term "significantly high yield", claim 19 has been amended to recite the phrase "as compared to an un-manipulated or wild-type microorganism". Applicants request reconsideration and withdrawal of the rejection of claim 19 under 35 USC §112, second paragraph as being indefinite.

Claims 27, 34 and 49 are also rejected as being "unclear in the recitation of 'panE1 nucleic acid sequence' and 'panE'".

The Examiner is correct in his interpretation of the terms "panE1" and "panE" as "a nucleic acid encoding a polypeptide having ketopantoate reductase enzymatic activity" as described in Example 2 (page 65). This is the meaning intended by Applicants.

Claim 49 is rejected as being "unclear in the recitation of 'coaX'".

The Examiner is correct in his interpretation that the term "coaX" is a "nucleic acid encoding a polypeptide having pantothenate kinase enzymatic activity. This is the meaning intended by Applicants. Moreover, "coaX" is a "nucleic acid encoding a polypeptide distinct from coaA.

In view of the above clairifcations made of record by the instant response, Applicants submit that the meaning of the terms, "panE" and "panE2" and "coaX" are clear. Reconsideration and withdrawal of the rejection of claims 27, 34 and 49 under 35 USC §112, second paragraph as being indefinite is respectfully requested.

Rejection of Claims 1, 2, 7, 12, 19, 24, 26-28, 33, 34, 49, and 51 Under 35 USC §112, First Paragraph

Claims 1, 2, 7, 12, 19, 24, 26-28, 33, 34, 49, and 51 are rejected under 35 USC §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The Examiner states that "[t]he scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the large number of microorganisms broadly encompassed by the claims". It is the Examiner's position that the present disclosure is "limited to a method for increased production of pantoate and pantothenate using a microorganism transformed with an expression vector comprising SEQ ID NO:29 encoding the *B. subtillis* ketopantoate reductase of SEQ ID NO:30, a method for increased production of pantoate and pantothenate using a microorganism transformed with an expression vector comprising a nucleic acid encoding an *E.coli* or *S.typhimurium* ketopantoate reductase and optionally wherein pantothenate is produced at a level of up to 2gram/liter, a method for increased production of pantoate and pantothenate using a microorganism transformed with an expression vector comprising the nucleic acid sequence of SEQ ID NO:29 encoding the *B. subtilis* ketopantoate reductase of SEQ ID NO"20, and a method for enhancing production of pantothenate using a *Bacillus* having a deletion of the *coaX* gene of SEQ ID NO:9, a microorganism transformed with an expression vector comprising a nucleic acid encoding an S176L

mutation in SEQ ID NO:2 or a Y181H mutation in the coaA gene product of SEQ ID NO:3 and co-transformed with an expression vector comprising a nucleic acid encoding an *E. coli* or *S. typhimurium* ketopantoate reductase. Based on a review of the factors set forth in *In re* Wands, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir, 1988), the Examiner concludes that '[t]he amount of necessary experimentation is not routine; due to lack of guidance and working example, and the high degree of unpredictability in the art...it is not routine in the art to screen for multiple modifications to a microorganism, nucleic acid or polypeptide".

The Examiner states that the specification teaches only a "single representative species of the genus of recited microorganisms" and the "scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the large number of microorganisms broadly encompassed by the claims".

Applicants respectfully traverse. The instant specification teaches at least, for example, at page 6, lines 4-24 that ketopantoate reductase overexpressing microorganisms can be generated by genetically manipulating said microorganisms to express greater levels of ketopantoate reductase that expressed prior to manipulation or as compared to a microorganism which has not been manipulated. The specification teaches, for example, introduction of exogenous genes as well as modification of the regulatory sequences of endogenous genes as a means of accomplishing such overexpression. The instant specification is replete with teachings as to how one would accomplish either of said types on overexpression. For example, the specification, at least at pages 13-17, as well as at least Examples II-III teach and exemplify expression of pantothenate biosynthetic enzymes (*e.g*, ketopantoate reductase) *via* introduction of the DNA encoding such enzyme on a plasmid including various regulatory elements. In particular, Example II exemplifies overexpression of the *B. subtilis panE* gene on a plasmid, with a new ribosomal binding site (RBS) and a new promoter. Example III further provides a working example of changing the regulation of endogenous pantothenate biosynthetic enzymes, in particular, by modifying the promotor sequences of the *panBCD* operon.

Applicants also submit that the isolation of orthologous genes from divergent organisms and the production of modified organisms is so routine in the art that multiple "cookbooks" of "recipes" with standard techniques are available (see *e.g. Manual of Industrial Microbiology and Biotechnology*. A. L. Demain and J.E. Davies (eds.), ASM Press. 1999; Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; *A Manual for Genetic Engineering: Advanced Bacterial Genetics*. Davies, R.W., Botstein, D., and Roth, J.R., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 1980). Furthermore, one skilled in the art can manipulate and overexpress genes from multiple microorganisms with the aid of kits and appropriate expression vectors that can be purchased from multiple vendors, including Stratagene (http://www.stratagene.com/index.asp?catId=22), and Clonetech (http://www.promega.com/vectors/allvectors.htm).

The Examiner also states that "one of high skill in the art would recognize the high degree of unpredictability in modifying a microorganism with the expectation of obtaining the desired result". "For example, Baigori, *et al.* teach mutagenesis of a *B. subtillis* strain resulting in a pantoate/pantothenate auxotroph with significantly reduced ketopantoate reductase activity" and that overexpression of ketopantoate reductase using an expression vector may result in decrease pantothenate production (see pages 68, lines 13-17). The Examiners objections are noted and respectfully traversed. The mutagenesis and identification of any microorganism with the desired phenotype (*e.g.* increased pantothenate production, is not relevant to the instant specification, since mutagenesis results in mutations (usually multiple mutations) in unrelated genes and that the claims are directed to methods of overexpressing a gene to produce pantothenate. Moreover, one skilled in the art is aware that the unexpected decrease in production of a compound can be easily overcome by choice of a different expression method, vector or strain carrying the gene of interest.

The Examiner is also objects to the claims by stating, that "it is not routine in the art to screen for multiple modifications to a microorganism, nucleic acid or polypeptide". Applicants respectfully traverse and submit that due to the nature of

microorganisms, *e.g.* their quick reproductive/growth cycles, growth as haploid and/or diploid organisms, need for limited growth space (*e.g.* hundreds of individual colonies can be screened for the appropriate phenotype on a single 100mm x 13mm Petri dish) and the availability of standard methods (described above), it is routine in the art and an effective method to screen for multiple mutations by screening hundreds to thousands of organisms to identify the novel strain with the desired phenotype.

In view of the extensive teachings and exemplification of methods for overexpressing either exogenous and/or endogenous biosynthetic enzyme encoding gene sequences, Applicants respectfully submit that the scope of claims (1, 2, 7, 12, 19, 24, 26-28, 33, 34, 49, and 51) is clearly enabled and respectfully requests reconsideration and withdrawal of the rejection under 35 U.S.C. 112, first paragraph.

Rejection of Claims 1, 2, 7, 12, 19, 24, 26, 27, 33, and 34 Under 35 USC §102(b)

Claims 1, 2, 7, 12, 19, 24, 26, 27, 33, and 34 are rejected under 35 USC §102(b) as being anticipated by Baigori, *et al.* (*J Bacteriol* (1991) 173:4240) and Frodyma, *et al.* (*J Biol Chem* (1998) 273:5572). The Examiner states that, "[w]hile Baigori, *et al.*. do not specifically teach their method results in the increased production of pantoate or pantothenate, production of these compounds would have inherently resulted from the overexpression of ketopantoate reductase in *Bacillus subtillis* as Baigori *et al.*. teach *Bacillus subtillis* has a pantothenate biosynthetic pathway. This anticipates claims 1, 2, 7, 19, 24, 26, 27, 33, and 34 as written". Furthermore, "[w]hile Frodyma, *et al.*. do not specifically teach their method results in the production of pantoate and pantothenate, the production of these compounds would have inherently resulted from the overexpression of *upbA* in *E. coli* as Frodyma, *et al.*. teach *E. coli* has a pantothenate biosynthetic pathway. This anticipates claims 7, 19, 24, and 26 as written".

Applicants respectfully traverse and submit that Baigori *et al.* teach the isolation and characterization of *Bacillus subtillis* chemically induced **mutants blocked in the synthesis of pantothenic acid**. These mutants are auxotrophic for pantothenate, *e.g.*, they are unable to produce pantothenate. They are dependent for growth on pantothenate feeding from the culture medium (page 4240, left column, second paragraph). Furthermore, Baigori, *et al.* teach that revertants of strain UR1 and UR2 show **normal**

levels of transferase and reductase activity (page 4240, right column, second paragraph). Nothing is disclosed about overexpression, which would lead to higher levels of transferase and reductase activity. The presumed higher activities disclosed on page 4241, Table 2, are not associated with transformants of the strains UR1 and UR2, as claimed by the Examiner. Rather, the somewhat higher over mutant levels of transferase and/or reductase activities are associated with **revertants** of the strains UR1 and UR2. These data indicate that the phenotype associated with the original mutant has now been spontaneously and naturally reversed to the wild-type phenotype by a second spontaneous mutation in an unidentified gene. Nothing is disclosed in Baigori *et al.* about a method for the production of pantothenate by overexpressing or recombining genes of the pantothenate biosynthsis chain. In fact, the instant invention is directly opposite to the teaching of Baigori, *et al., e.g.* a method to produce pantothenate that involves **overexpressing** the *panE* gene product, and therefore Baigori, *et al.* is not relevant in view of novelty and obviousness.

In fact, the methodology described in Baigori *et al.* for producing *panE* mutants would not result in the successful production of such mutants for the following reasons. Baigori, *et al.* claim to have isolated Pan$^-$ mutants of *B. subtilis* and have characterized the mutations as being in either the *panB* or in the *panE* genes. The classification of the mutations was based on measuring a reduction in PanB and PanE enzyme activities. Baigori *et al.* also claim to have mapped the mutations to the *purE-tre* region of the *B. subtilis* chromosome, and the mutations are described as being close to each other.

Also, the work of Baigori, *et al.* teaches against finding the *panE* gene because it indicated that the gene was located in a different region of the genome (60°) when in fact, the gene is located at (135°).

Even if one were to assume arguendo that Baigori, *et al.* had described such a microorganism, Applicants respectfully submit that the reference would fall significantly short of teaching methods of producing panto-compounds comprising culturing a microorganism that expresses ketopantoate reductase *and* a second pantothenate biosynthetic enzyme as nothing in the reference teaches or even remotely suggests overexpressing such enzymes either alone or in combination to achieve the claimed production methods. Furthermore, if one were to assume arguendo that the methods of

Baigori *et al.* were to result in a retransformed *panE* mutant having normal ketopantoate reductase activity (as recited in the sole statement referring to such a microorganism), Applicants respectfully submit that such microorganisms (*e.g.*, reverted to "wild type" ketopantoate reductase levels) would fail to produce at least 0.2 g/L panto-compound, as recited in amended claims 14-20, 23 and 25-27 (see *e.g.*, the instant specification at Table 6 showing that parent *Bacillus* strains, for example RL-1 and PY79, having "normal" ketopantoate reductase levels produce significantly lower concentrations of panto-compound).

With regard to Frodyma, *et al.*., they teach the production and purification of the *apbA* gene product (previously designated *panE*), ketopantoic acid reductase, by overexpression of that gene in ***Salmonella typhimurium*** (page 5572, right column, first paragraph). Frodyma, *et al.* do not teach any method for the production of panto-compounds by overexpressing at least one ***Bacillus*** pantothenate biosynthetic enzyme as in claim 1. Furthermore, Frodyma, *et al.* do not disclose a β-alanine independent high yield production method for pantothenate as in claim 19, or a method of producing pantothenate in a manner independent of precursor feed as in claim 14. Since claims 24 and 26 are dependent on the aforementioned claims (14 and 19) they have the same limitations. Therefore, Frodyma, *et al.* do not anticipate the invention in the present specification, *e.g.* a method to produce pantothenate that involves overexpressing the *Bacillus panE* gene product and a method to produce pantothenate in a manner independent of feed. Furthermore, nothing in Frodyma *et al.* teaches or even remotely suggests overexpressing a second pantothenate biosynthetic enzyme in addition to the *apbA* gene product. Therefore Frodyma *et al.* does not teach the inventive process and fails to anticipate the claimed invention.

For at least the reasons set forth above, Applicants respectfully submit that Baigori *et al.* and Frodyma, *et al.* fail to teach the claimed inventive process and therefore fails to anticipate the claimed invention. Accordingly, reconsideration and withdrawal of the rejection of claims 1, 2, 7, 12, 19, 24, 26, 27, 33, and 34 under 35 U.S.C. § 102 is requested.

Rejection of Claims 1, 2, 7, 12, 19, 24, 26, 27, 33, and 34 Under 35 USC §103(a)

Claim 12 is rejected under 35 USC §103(a) as being unpatentable over Frodyma *et al.* in view of Hikichi, *et al.* (US patent 5, 518, 906). The Examiner states that Frodyma, *et al.* teach *panE* encoding ketopantoate reductase is a gene of the pantothenate biosynthetic pathway and Hikichi, *et al.* teach a method for the production of pantoate and pantothenate using an *E. coli* host transformed with plasmid pFV31 comprising *E. coli panB*, *panC* and *panD* genes encoding ketopnatoate hydroxymethyltransferase, pantothenate synthase and aspartate-decarboxylase, respectively. Hikichi, *et al.* teaches pantothenate is a useful vitamin substance and that pantoate is useful as an important intermediate for synthesis of pantothenate and CoA. Hikichi, *et al.* teach that strain transformed with a plasmid carrying genes involved in the biosynthesis of pantothenate accumulates increases levels of pantothenate in the culture medium". Therefore, "[a]t the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Frodyma, *et al.*. and Hikichi, *et al.*. for a method for producing pantothenate using the host cell of Frodyma, *et al.*. overexpressing ketopantoate reductase additionally overexpressing *panB*, *panC*, and *panD* genes of Hikichi, *et al.*.".

Applicants respectfully traverse. Hikichi, *et al.* teach a method of producing D-pantothenic acid or a salt thereof characterized by bringing a microbe belonging to the family Enterobacteriacceae having resistance to salicylic acid and capable of producing D-pantothenic acid in the presence of β-alanine (see abstract). Furthermore, Hikichi, *et al.* teach that preferably the microbes are transformed with a plasmid DNA comprising the genes panB, panC, and panD (see claims 5 and 6). Nothing is disclosed about a process for the production of panto-compounds by overexpressing the ketopantoate reductase gene (*panE*) and at least one other pantothenate biosynthetic enzyme. Nothing is disclosed in the present specification about microbes possessing a resistance against salicyclic acid. Therefore, Hikichi, *et al.* can not make the present invention obvious.

Frodyma, *et al.* teach the identification of the enzymatic function of *apbA* (*panE*). Nothing is disclosed about the overexpression of *panE* **for the production of pantothenic acid**. Hence, the present invention is not obvious in the light of the teachings of Frodyma, *et al.* Neither in Frodyma, *et al.* or in Hikichi, *et al.* are there

indications that the skilled worker should combine the two references. To combine the two references is only possible once the methods of the present invention are known. Therefore, the method to produce a panto-compound comprising culturing a ketopantoate reductase and at least one pantothenate biosynthetic enzyme over-expressing microorganism is not obvious.

Furthermore, in order to create an obviousness rejection under 35 USC §103(a), three requirements must be met according to case law. First, the prior art relied upon, coupled with the knowledge generally available in the art at the time of the invention, must contain some suggestion or incentive that would have motivated the skilled person to modify a reference or to combine references. See In re Fine, 837 F.2d 1071, 1074, 5 U.S.P.Q. 2d 1596, 1598 (Fed Cir. 1988) and In re Skinner, 2 U.S.P.Q. 2d 1788, 1790 (Bd. Pat. App. & Int. 1986). No such motivation can be drawn from Frodyma, *et al.*, Hikichi, *et al.* or the combination of the two.

Second, the proposed modification or combination of references must teach or suggest all limitations of the claims. See In re Wilson, 424 F. 2d 1200, 1209, 18 U.S.P Q. 2d 1016, 1023 (Fed. Cir. 1991) and In re Erlich, 3 U.S.P.Q. 2d 1011, 1016 (Bd. Pat. App. & Int. 1986).

Third, the prior art reference or combination of references must teach or suggest all limitations of the claims. See In re Wilson, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494, 496 (C.C.P.A. 1970). And the teachings or suggestions, as well as the expectation of success, must come from the prior art, not Applicants disclosure. See In re Vaeck, 947 F.2d 488, 20 U.S.P.Q. 2d 1438, 1442 (Fed. Cir. 1991). As Hikichi, *et al.* does not disclose ketopantoate reductase gene for the production of panto-compounds and Frodyma, *et al.* is related to the identification of the enzymatic function of *apbA* gene, there is no motivation for the skilled worker to combine both references.

Moreover, when viewing the problem to be solved as producing significant or substantial quantities of panto-compounds (*e.g.*, pantoic acid and/or pantothenic acid) using engineered microorganisms, Hikichi *et al.* teach that the solution involves overexpressing the *panBCD* operon in, for example, salicylic acid resistant mutants, but is silent as to any need or desire to isolate and/or overexpress any additional pantothenate biosynthetic enzymes. Frodyma *et al.* teach a role for the *Salmonella typhimurium apbA*

gene product in pantothenate biosynthesis but fails to teach or even remotely suggest overexpressing this gene product in combination with a second pantothenate biosynthetic enzyme for the production of significant concentrations of panto-compounds. The fact that *panE* plays a role in pantothenate production and that pantothenate is an important vitamin substance are accurate factual statements recited not only in the cited references but well known at the time of filing of the instant invention but, at best, characterize the problem to be solved by the instant invention rather that teach or even remotely suggest the solution provided by Applicants' invention. As the Examiner is aware, it is improper to reconstruct the patentee's claimed invention from the prior art by using the patentee's claim as a "blueprint." When prior art references require selective combination to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight obtained from the invention itself. *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 227 USPQ 543 (Fed. Cir. 1985), see also M.P.E.P. §2141. In view of the above comments, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 11-13 in view of Frodyma *et al.* in view of Hikichi *et al.* Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-4 under 35 U.S.C. 102(b).

Claims 49 and 51 are rejected under 35 USC §103(a) as being unpatentable over Baigori, *et al.* or Frodyma, *et al.* in view of Hikichi, *et al.* as applied to claim 12 above, and further in view of Vallari, *et al.*. (*J Bacteriol* (1988) 170:3961) and Leung (Coenzyme-A Technologies Inc., Technical Articles and Scientific Research, "Coenzyme-A 'The Master Coenzyme'"). The Examiner states that "[a]t the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Frodyma, *et al.*., Hikichi, *et al.*., Vallari, *et al.*., and Leung for a method producing CoA using the host cell of Vallari, *et al.*. expressing the mutant pantothenate kinase and additionally co-expressing the ketopentoate reductase of Frodyma, *et al.*. and the panB, panC, and panD genes of Hikichi, *et al.*. One would have been motivated for a method for producing CoA using the mutant pantothenate kinase and additionally co-expressing the ketopentoate reductase of Frodyma, *et al.* and the panB, panC, and panD genes of Hikichi, *et al.* because the teachings of Hikichi, *et al.*. who teaches a strain

transformed with a plasmid carrying genes involved in the biosynthesis of pantothenate accumulates increased levels of pantothenate in the culture medium and that pantothenate is useful as an important intermediate for synthesis of CoA, Vallari, *et al.* who teach CoA is an essential cofactor in numerous metabolic pathways, and Leung who teach the beneficial effects of CoA supplementation".

Leung teach that CoA is an important co-factor in both fatty acid metabolism and sex hormone synthesis and based on his hypothesis is important in the control of acne vulgaris; both points are immaterial to the present specification.

Vallari, *et al.* teach that the regulation of pantothenate kinase is "the most important determinant in CoA biosynthetic rate" and the isolation of *E. coli* mutants with inhibition of the negative feedback mechanism of CoA to pantothenate kinase leading to significantly elevated levels of CoA in the microbes. They do not specifically target any of the genes in the pathway of panto-compound production for mutation; rather they induce mutations by P1 bacteriophage transduction, which is a random process of mutagenesis, and therefore have no reason to know whether there was a specific mutation in any of the genes necessary for panto-compound production. In contrast, the present invention describes the specific isolation of the *coaX* gene (see Example XIV), which is defined as "a second pantothenate kinase-encoding gene", *e.g.*, in "*B. subtilis*". This gene complements an *E. coli* mutant that contains a temperature sensitive pantothenate kinase and is not related by homology to any previously known pantothenate kinase gene". Furthermore, if Vallari, *et al.* believe that pantothenate kinase is "the most important determinant in CoA biosynthetic rate", there would be no motivation improve that production and thus to combine any of the teachings of Frodyma, *et al.* who teach the purification of ketopantoic acid reductase, and Hikichi, *et al.* who teach a method of producing D-pantothenic acid in the presence of β-alanine. Applicants respectfully request that for at least the foregoing reasons, the rejection of claims 49 and 51 under 35 USC §103(a) be reconsidered and withdrawn.

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Group Art Unit: 1652
Examiner: D Steadman

CONCLUSION

In view of the above amendments and remarks, it is believed that this application is in condition for allowance. If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

Respectfully submitted,

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